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EFFECT OF USING FLAX MEAL IN BROILER DIETS ON MEAT FATTY ACIDS CONTENT

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Abstract

The paper aimed to present the effect of using two by-products from food industry in broiler diets, on meat fatty acids content. A 4-week feeding trial (14-42 days) was conducted on 200, Cobb 500 broilers, assigned to 5 groups (C, E1, E2, E3, E4) and reared in floor pens. The structure of all five groups was enriched in polyunsaturated fatty acids by (PUFA) using 4% flax meal, characterized by 50.73% alpha-linolenic acid (ALA). Compared to the control formulation, in both phases (grower/finisher) the experimental formulations included 3% and 6% white (2.48% ALA) or red (1.51% ALA) grape pomace. At the end of the trial, 6 broilers/ group were slaughtered and samples of thigh and breast meat were collected in order to determine the fatty acids content. The obtained results showed a higher content of PUFA for the experimental diets which included grape pomace, compared to the one that had only flax meal. Regarding the meat samples, the experimental diet that was supplemented with 3% red grape pomace recorded the highest content of PUFA, both in thigh and breast meat, compared to the control diet.

Key words*: broiler, fatty acids, flax meal, meat.*

INTRODUCTION

It is estimated that by 2050 the world's population will reach 9 billion, and implicitly global demand for food will grow steadily in the near future (Wang et al., 2016). The meat fatty acids content is an important quality parameter, especially regarding the possible impact on human health following the chicken meat consumption (Rahimi et al., 2011). Omega-3 polyunsaturated fatty acids (PUFA n-3) play an important role in the human diet, their health benefits including lower risk of cancer, cardiovascular disease, skin disorders, anxiety and stress, obesity, inflammation, arthritis rheumatoid, osteoporosis, asthma and allergies (Roy et al., 2020). Chicken meat is healthier, with relatively low-fat content, compared to meat from other animals. The problem most countries face is the high intake of omega-6 fatty acids in diet, compared to omega-3, which leads to pathological changes (Simopoulos, 2016). Due to the limited level of PUFA n-3 in the human diet, different strategies have been tried lately to obtain food of animal origin with a higher concentration in these fatty acids. The polyunsaturated fatty acids are recognized as essential constituents for normal growth and development in both humans and animals'nutrition (Zhang et al., 2010). Therefore, current nutritional strategies are focused on assessing the effects of terrestrial sources rich in PUFAs in poultry feed and their subsequent implications on product quality. Hybridity, sex, body weight at slaughter of chickens and not least the quality of the feeds are factors that can influence the meat quality (Pop and Frunza, 2018).

Flax meal is one of the by-products that can be successfully introduced into broiler diets in order to improve feeds and meat nutritional value, as well as their sensory and functional properties (Russ and Oreopoulou, 2007). This by-product is rich in fatty acids, their PUFA n-6: n-3 ratio being subunit. A high-fatty acids diet is an important source of energy, plus a better adsorption of fat-soluble vitamins and all the nutrients in the diet (Alzueta et al., 2003). On the other hand, the lack of antioxidants causes chicken meat to have a short shelf life, being prone to degradation due to lipid peroxidation.

Grape pomace is rich, particularly in a wide range of polyphenols, which act as powerful antioxidants (Brenes et al., 2008). Studies regarding the grape pomace using in broilers diet indicated its effectiveness in preventing or at least delay the meat lipid peroxidation (Khodayari et al., 2014). The fact that these by-products are a cheap source of antioxidant compounds offers important economic benefits when used in poultry feed.

This study was, therefore, conducted to determine the effects of supplementing broilers PUFA enriched diets with two varieties of grape pomace on production performances, the development of carcass and organs and meat fatty acids content.

MATERIALS AND METHODS

Birds and experimental design

The 4-week feeding trial was conducted within the experimental halls of the National Research and Development Institute for Biology and Animal Nutrition (IBNA-Balotesti, Romania) according to the protocol approved by the Ethical Commission of the institute. A number of 200, Cobb 500 broiler chicks, aged 14 days, were weighed individually and assigned to five groups C, E1, E2, E3 and E4 (40 chicks per group), without significant differences ($p>0.05$) of body weight between groups. The chicks were housed in environmentally controlled conditions to the ground in a semi-intensive system, according to Cobb 500 Management Guide.

During the grower (14-28 days) and finisher (29-42 days) stages, the chicks from control group (C) received a conventional diet based on corn and soybean meal (Table 1). The structure of all five groups was enriched in PUFA by using 4% flax meal. Compared to the control diet (C), the experimental formulations have differentiated by the addition of two levels and two varieties of grape pomace: 3% (E1) and 6% (E2) white grape pomace (Tamaioasa Romanesca); 3% (E3) and 6% (E4) red grape pomace (Merlot) variety. The broilers had free access to the feed and water.

Throughout the experimental period (14-42 days) the following parameters were monitored: body weight (g), average daily feed intake (g feed/broiler/day), average daily weight gain (g/broiler/day) and feed conversion ratio (g feed/g gain).

Sampling and chemical analysis

Flax meal was analysed for the fatty acids content and grape pomace was analysed in order to determine the antioxidant capacity and total polyphenol concentration. Compound feed samples were collected and assayed for the fatty acids content and regarding the evolution of fat degradation indices after 7 and 14 days from manufacturing. At the end of the feeding trial, 6 broilers/group were slaughtered according to the working protocol and samples of breast and thigh meat (n = 6 for each dietary treatments) were collected and stored at −20°C until the fatty acid content was analysed.

Table 1. Diet formulation

Diet composition, %	Grower stage (14-28 days)					Finisher stage (29-42 days)				
	C	E1	E2	E3	E4	C	E1	E2	E3	E4
Corn	35.50	31.37	37.16	31.60	37.36	39.37	34.23	31.12	34.46	31.97
Wheat	20.00	20.00	10.00	20.00	10.00	20.00	20.00	20.00	20.00	19.58
Corn gluten	4.00	6.00	9.25	6.00	8.73	6.00	6.00	10.00	6.00	10.00
Soybean meal	27.02	25.02	22.50	24.86	22.83	20.81	21.32	16.81	21.16	16.53
Flax meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
White grape pomace	0.00	3.00	6.00	0.00	0.00	0.00	3.00	6.00	0.00	0.00
Red grape pomace	0.00	0.00	0.00	3.00	6.00	0.00	0.00	0.00	3.00	6.00
Vegetable oil	4.21	5.21	5.50	5.13	5.50	4.67	6.29	0.68	6.21	6.50
Monocalcium phosphate	1.54	1.56	1.65	1.56	1.65	1.45	1.45	1.49	1.45	1.49
Calcium carbonate	1.40	1.41	1.40	1.41	1.40	1.33	1.32	1.34	1.32	1.35
Salt	0.36	0.36	0.37	0.36	0.37	0.33	0.34	0.34	0.34	0.34
Methionine	0.29	0.28	0.24	0.28	0.25	0.26	0.27	0.22	0.27	0.23
Lysine	0.41	0.47	0.55	0.48	0.54	0.48	0.47	0.60	0.48	0.61
Threonine	0.22	0.27	0.33	0.27	0.32	0.25	0.26	0.35	0.26	0.35
Choline	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[1]Content per kg diet: 1100000 IU vitamin A; 200000 IU vitamin D3; 2700 IU vitamin E; 300 mg vitamin K; 200 mg vitamin B1; 400 mg vitamin B2; 1485 mg pantothenic acid; 2700 mg nicotinic acid; 300 mg vitamin B6; 4 mg vitamin B7; 100 mg vitamin B9; 1.8 mg vitamin B12; 2000 mg vitamin C; 8000 mg manganese; 8000 mg iron; 500 mg copper; 6000 mg zinc; 37 mg cobalt; 152 mg iodine; 18 mg selenium.

The grape pomace total polyphenol concentration was determined according to the method described by Untea et al. (2020) using a spectrophotometer. The results were expressed in mg gallic acid equivalents/g sample (mg EAG/g sample).

The antioxidant capacity of grape pomace was determined according to the phosphomolybdenum method described by Untea et al. (2020). The results were expressed in mmol vitamin E equivalent/g sample.

Fat degradation indices of feed samples. The peroxide value was determined using the iodometric method, according to SR EN ISO 3960:2017 and the results were expressed in ml thiosulfate 0.1 N/g fat. Fat acidity was determined using the volumetric method, according to ISO 660:2009 and the results were expressed in mg KOH/g fat.

The fatty acids were determined by gas chromatography, according to standard SR CEN ISO/TS 17764 -2: 2008, using a Perkin Elmer-Clarus 500 gas chromatograph, with capillary column injection system, high polarity stationary phase (BPX70: 60 m x 0.25 mm inner diameter and 0.25 μm thick film), and high polarity cyanopril phase, which give similar resolution for different geometric isomers (THERMO TR-Fame: 120 m x 0.25 mm ID x 0.25 μm film). The results were expressed in g FAME/100 g total FAME.

Statistical analysis

The analytical data were compared by variance analysis (ANOVA and t test), using StatView program. The experimental results were expressed as mean values, the differences being considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSIONS

The flax meal had a content of 66.72% total PUFA, of which 50.73% were represented by PUFA n-3 and 15.99% PUFA n-6, resulting an ideal value of their ratio of 0.31. The white grape pomace was characterized by an antioxidant capacity of 264.46 mmol vitamin E equivalent/g and a total polyphenol concentration of 13.13 mg GAE/g. The red grape pomace had an antioxidant capacity of 213.69 mmol vitamin E equivalent/g and a total polyphenol concentration of 21.05 mg GAE/g. The results regarding the fatty acid content of compound feed are shown in Table 2. As can be seen, the highest ALA content in the grower stage, compared to the control group, was obtained in the 3% grape pomace level groups. In the finisher stage, the ALA content increased in the groups with a higher level of grape pomace (6%). Regarding the PUFA level, there was an improvement of feed in all experimental groups, compared to group C, regardless of the inclusion level.

Table 2. Fatty acids content of the compound feeds by level of unsaturation

Groups	ALA, g/ 100 g total FAME	∑ SFA, %	∑ MUFA, %	∑ PUFA, %
	Grower stage (14-28 days)			
C	8.98	15.36	24.90	59.23
E1	9.26	14.83	23.40	61.23
E2	8.79	15.13	23.31	61.15
E3	9.29	14.91	23.88	61.12
E4	9.14	14.85	23.98	61.11
	Finisher stage (29-42 days)			
C	8.78	14.91	24.97	60.07
E1	8.58	14.92	24.22	60.87
E2	8.84	11.12	26.73	61.61
E3	8.79	10.96	28.48	60.38
E4	8.90	11.19	26.19	62.14

ALA-alpha linolenic acid; ∑-sum; SFA-saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids.

Periodically, the evolution of fat degradation indices from feeds was monitored, making determinations regarding their degradation rates (Table 3).

Table 3. The evolution of the fat degradation indices of the compound feed

Fat degradation indices		*Grower stage (14-28 days)*				
		C	E1	E2	E3	E4
Peroxide value (ml thiosulfate 0.1 N/g fat)	7D	0.25	0.26	0.28	0.30	0.28
	14 d	0.54	0.56	0.62	0.65	0.54
Fat acidity (mg KOH/g fat)	7 d	12.10	13.05	13.70	13.48	13.05
	14 d	14.41	15.10	15.62	15.38	15.06
		Finisher stage (29-42 days)				
Peroxid value (ml thiosulfate 0.1 N/g fat)	7 d	0.26	0.29	0.30	0.33	0.29
	14 d	0.59	0.60	0.58	0.62	0.61
Fat acidity (mg KOH/g fat)	7 d	12.28	12.70	12.59	12.69	12.83
	14 d	15.46	15.95	15.97	15.28	16.01

Analyses carried out both after 7 days and after 14 days from feeds manufacturing and storage, showed that the combined feeds did not degrade. The values obtained for peroxide index and fat acidity were within the allowed standardize limits.

The production parameters recorded during the entire experimental period are shown in Table 4. Significant differences ($p<0.05$) were observed regarding the final weigh, results corroborated withaverage daily weight gain of the broilers from all experimental groups, compared to group C.

Table 4. Production parameters throughout the experimental period (14-42 days)

Groups	Initial weight (g)	Final weight (g)	Average daily weight gain (g/broiler/day)	Average daily feed intake (g feed/broiler/day)	Feed conversion ratio (g feed/g gain)
C	506.54[a]	3333.10[a]	78.27[a]	120.85[a]	1.54[a]
E1	506.51[a]	3163.45[bc]	74.20[b]	115.49[a]	1.56[a]
E2	506.91[a]	3186.31[b]	74.75[b]	114.19[a]	1.53[a]
E3	506.85[a]	3109.76[bc]	72.93[bc]	113.54[a]	1.56[a]
E4	506.33[a]	2987.63[c]	70.03[c]	109.75[a]	1.56[a]
SEM	3.192	23.080	0.550	3.982	0.025
p Value	>0.9999	<0.0001	<0.0001	0.9379	0.9112

[a-c]Means within a column with no common superscript differ ($p<0.05$).

The broilers with the lowest weight at the end of the feeding trial were those fed with 6% red grape pomace. There was no significant ($p>0.05$) difference in feed conversion throughout the entire experimental period, compared to C group. Kumanda et al. (2019) evaluated the effect of different grape pomace level (2.5%; 4.5%; 5.5% and 7.5%) in Cobb 500 broilers diet. The use of 7.5% grape pomace level had the lowest feed intake ($p<0.05$) but there were no dietary effects on weight gain. As well, in the same broilers group, they had the lowest ($p<0.05$) feed conversion ratio (1.45) compared to the control group (1.79). Abu Hafsa and Ibrahim (2017) investigated the effect of using different levels of grape flour (10; 20 and 40 g/kg feed) on Cobb 500 broilers growth performance during 42 day. The reported data showed that 20 g of grape flour/kg of feed led to the broilers weight gain, obtaining higher final weights and improved the feed conversion ratio, without affecting the feed consumption.

The measurements made at broilers laughter regarding the development of their carcass and organs (Table 5) revealed a smaller ($p<0.05$) carcass for of broilers the fed with 6% white grape pomace, compared to the control. Regarding the breast meat weight, although in group E1 it was registered an increase ($p>0.05$) by 1.48% compared to C group, the broilers fed with red grape pomace showed significant smaller ($p<0.05$) weights. Significant differences ($p<0.05$) for thigh meat weight were obtained only for broilers fed with 3% white grape pomace (E1) and 6% red grape pomace (E4). There were no significant differences ($p>0.05$) between groups in terms of spleen, bile and gizzard samples. An increase ($p<0.05$) with 12.08% for the liver weight was observed in E2 group, compared to the control group. In contrast, the weight of heart samples from E2, E3 and E4 groups recorded smaller ($p<0.05$) values compared to those from the control group.

Table 5. Data regarding the development of carcass and organs (42 days-old broilers)

Parameters (g/broiler)	C	E1	E2	E3	E4	SEM	*p* Value
Carcass	2619.17[a]	2590.00[a]	2607.50[a]	2528.33[a]	2340.83[b]	23.177	<0.0001
Breast	756.00[a]	767.19[a]	727.81[ab]	690.87[bc]	653.14[c]	11.723	0.0036
Thigh	570.60[a]	508.42[b]	548.75[a]	560.10[a]	494.15[b]	8.053	0.0016
Liver	59.14[ab]	55.90[a]	66.29[b]	57.67[ab]	55.31[a]	1.515	0.0300
Spleen	3.37[a]	2.90[a]	3.44[a]	3.06[a]	2.84[a]	0.114	0.3499
Bile	2.48[a]	2.15[a]	2.15[a]	2.35[a]	2.54[a]	0.134	0.8185
Heart	16.49[a]	14.61[ab]	13.85[bc]	13.38[bc]	12.64[c]	0.370	0.0053
Gizzard	35.28[a]	33.41[a]	36.98[a]	38.04[a]	33.82[a]	1.168	0.7037

[a-c] Means within a raw with no common superscript differ ($p<0.05$).

The results regarding the fatty acid content of the thigh meat samples are shown in Table 6. Significant differences ($p<0.05$) compared to the control group were observed for all fatty acids'classes according to the level of unsaturation. There was an increase ($p<0.05$) of PUFA content particularly in groups with 6% white grape pomace (E2) and 3% red grape pomace (E3), compared to C group. However, there was no improvement in the PUFA n-6: n-

3 ratio. The obtained data are in agreement with those of Chamorro et al. (2015), in a study on the effect of grape meal in broilers diet (5% and 10%). They recorded an increase in the thigh meat PUFA content of 47.40 g PUFA for broilers fed with 5% grape meal and 53.10 g/100 g total fatty acids for broilers fed with 10% grape meal.

Table 6. Fatty acids content of the thigh meat according to the level of unsaturation

Fatty acids, %	∑SFA	∑MUFA	∑PUFA	Ω6/Ω3
C	25.76^{a}	31.88^{ab}	41.55^{a}	7.97^{a}
E1	24.24^{bc}	33.48^{ac}	41.74^{a}	10.99^{b}
E2	25.38^{ab}	30.75^{b}	43.42^{a}	8.64^{a}
E3	23.62^{c}	32.48^{abc}	43.51^{a}	9.82^{c}
E4	24.22^{bc}	34.12^{c}	41.36^{a}	10.08^{c}
SEM	0.258	0.35	0.466	0.242
p Value	0.0389	0.0082	0.4207	<0.0001

SFA-saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids.
$^{a-c}$ Means within a column with no common superscript differ ($p< 0.05$).

The breast meat fatty acids (Table 7) highlighted as well as the thigh meat an improvement of PUFA content for all 4 experimental groups. It was maintained the same tendency, namely the better efficiency of diets administered in E2 and E3 groups. Another favourable aspect of grape pomace using in broilers diet was that decreased ($p<0.05$) SFA content in breast meat samples, particularly for broilers fed with red grape pomace (E3). The obtained results are similar to those reported by Olteanu et al. (2017), who studied the effect of using 3% grape seed meal on meat fatty acid profile. Significantly higher values ($p<0.05$) were recorded in PUFA content from breast (32.46 ± 0.87 g) and thigh meat (37.68 ± 2.07 g), compared to the control group.

Table 7. Fatty acids content of the breast meat according to the level of unsaturation

Fatty acids, %	∑SFA	∑MUFA	∑PUFA	Ω6/Ω3
C	26.70^{a}	33.11^{ac}	39.83^{ab}	7.71^{a}
E1	25.24^{a}	35.79^{b}	38.82^{b}	10.12^{b}
E2	25.46^{a}	31.25^{ac}	43.09^{a}	8.14^{a}
E3	23.35^{b}	33.28^{c}	43.12^{a}	9.64^{b}
E4	25.62^{a}	37.82^{b}	36.34^{b}	11.19^{c}
SEM	0.309	0.537	0.706	0.271
p Value	0.0061	<0.0001	0.0024	<0.0001

SFA-saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids.
$^{a-c}$Means within a raw with no common superscript differ ($p<0.05$).

Modern human nutrition aims to reduce or completely exclude foods that contribute to the onset of diseases of this century, which leads to the involvement of researchers in a series of experiments aimed at obtaining foods with beneficial effects for human health (Pogurschi et al., 2010).Meat quality is a very important aspect for both producers and consumers, but especially for the latter. There are a number of factors that sum up the concept of meat quality, including flavour, appearance and texture, meat colour, and last but not least, the polyunsaturated fatty acids content (Banaszak et al., 2020). The manipulation of PUFA content in chicken meat through animal nutrition is currently the best strategy to obtain a healthy food product, which will provide consumers with the necessary nutrients and which will help to maintain their health, if not to prevent the diseases appearance. Therefore, the use of by-products from industry, which have high nutrients concentrations, can effectively contribute to improve the feeds chemical composition used in broilers diet and their meat quality, using natural sources of nutrients. In addition to capitalizing on the resources of existing raw materials and the economic efficiency achieved, this action is also a good way to solve the ecological problems, generated by the huge quantity of resulting residues.

CONCLUSIONS

The production parameters recorded throughout the experimental period showed that grape pomace influenced the average daily weight gain and therefore body weight, meaning that broilers gained less weight especially in the group with 6% red grape pomace.
On the other hand, the results of this study highlighted the beneficial effect of using flax meal in broilers diet in order to enrich them in polyunsaturated fatty acids. At the same time, the inclusion of grape pomace as natural antioxidant in diets contributed to overall quality of feeds and meat samples (thigh and breast) by slowing down the lipid degradation processes.
The obtained results showed a higher content of PUFA for the experimental diets which included grape pomace, compared to the one

that had only flax meal. Regarding the meat samples, the experimental diet that was supplemented with 3% red grape pomace recorded the highest content of PUFA, both in thigh and breast meat, compared to the control diet.

Thus, using flax meal and grape pomace as naturals feed ingredients has the potential to enhance the broilers production parameters and meat polyunsaturated fatty acids content.

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